FEBS Letters 584 (2010) 33–38

FEBS Letters

journal homepage: www.FEBSLetters.org

c-Abl tyrosine kinase interacts with MAVS and regulates innate immune response

Ting Song [a,1], Congwen Wei [a,1], Zirui Zheng [b,1], Yang Xu [b], Xiaozhong Cheng [a], Yuan Yuan [a], Kai Guan [a], Yanhong Zhang [a], Qingjun Ma [a], Wei Shi [b,*], Hui Zhong [a,*]

[a] State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Biotechnology, Room 841, East District Building, No. 27 Taiping Road, Haidian District, Beijing 100850, China
[b] Key Laboratory for Molecular Enzymology and Engineering, Ji Lin University, Changchun 130021, China

ARTICLE INFO

Article history:
Received 29 September 2009
Revised 6 November 2009
Accepted 8 November 2009
Available online 13 November 2009

Edited by Giulio Superti-Furga

Keywords:
c-Abl non-receptor tyrosine kinase
Mitochondrial antiviral signaling
Innate immune response
RIG1 signaling

ABSTRACT

The tyrosine kinase, c-Abl, plays important roles in many aspects of cellular function. Previous reports showed that c-Abl is involved in NF-κB signaling. However, the functions of c-Abl in innate immunity are still unknown. Here we demonstrate that the mitochondrial antiviral signaling (MAVS) protein can be physically associated with c-Abl in vivo and in vitro. MAVS interacted with c-Abl through its Card and TM domain. A phosphotyrosine-specific antibody indicated that MAVS was phosphorylated by c-Abl. Functional impairment of c-Abl attenuated MAVS or VSV induced type-I IFN production. Importantly, c-Abl knockdown in MCF7 cells displayed impaired MAVS-mediated NF-κB and IRF3 activation. Taken together, our results suggest that c-Abl modulates innate immune response through MAVS.

Structured summary:
MINT-7297498, MINT-7297511, MINT-7297557, MINT-7297574: *MAVS* (uniprotkb:Q7Z434) *physically interacts* (MI:0915) with *c-Abl* (uniprotkb:P00519) by *anti tag coimmunoprecipitation* (MI:0007)
MINT-7297542: *c-Abl* (uniprotkb:P00519) *physically interacts* (MI:0915) with *MAVS* (uniprotkb:Q7Z434) by *anti bait coimmunoprecipitation* (MI:0006)
MINT-7297526: *c-Abl* (uniprotkb:P00519) *physically interacts* (MI:0915) with *MAVS* (uniprotkb:Q7Z434) by *far western blotting* (MI:0047)

1. Introduction

c-Abl is a Src-like non-receptor protein kinase (PTK) ubiquitously expressed and plays important roles in regulating cell proliferation, apoptosis, adhesion, cell migration and stress responses [1–3]. The c-Abl PTK is localized both in the nucleus and in the cytoplasm and its kinase activity is tightly regulated. The existence of C-terminal DNA-binding motifs and nuclear localization signals in c-Abl enables shuttling between cytoplasmic and nuclear compartments, extending the exposure to additional Abl kinase substrates [4–6]. Studies suggest that nuclear c-Abl inhibits IκB-α degradation by inducing its phosphorylation, which eventually led to NF-κB inactivation. On the contrary, cytoplasmically localized Bcr-Abl can in fact potently activate NF-κB, which has been demonstrated to be essential for Bcr-Abl to induce transformation [7,8]. However, the functions of c-Abl in innate immunity are still unknown.

The innate immune system which characterized by the production of type-I interferon (IFN)-α/β cytokines and the activation of natural killer (NK) cells plays important roles in invading pathogens detection and elimination [9,10]. The host senses viral and bacterial pathogen invasion by recognition of pathogen-associated molecular patterns (PAMPs) with pattern recognition receptors (PRRs) including membrane bound toll-like receptors (TLRs) [11,12] and cytosolic sensory molecules such as the multi-domain containing NOD proteins and RIGI and MDA5 helicase [13–15]. Both RIG-I and MDA5 contain caspase recruitment domains (CARDs) that interact with the CARD domain-containing protein MAVS upon binding to uncapped RNA, resulting in MAVS association with IKK proteins [16–20]. MAVS association with IKKα/β activates NF-κB while association with IKKε leads to activation of IRF3. Coordinated activation of NF-κB and IRF3 pathways leads to the assembly of a multiprotein enhancer complex which drives expression of IFN-β and IFN-mediated antiviral immunity [21–23].

In this report, we show that the c-Abl tyrosine kinase associates with MAVS. Additionally, we show that depletion of c-Abl with small interference RNA or chemical inhibitor leads to decreased MAVS-induced IFN-β production, NF-κB and IRF3 activation.

* Corresponding authors. Fax: +86 10 66931809.
E-mail addresses: shiw@jlu.edu.cn (W. Shi), towall@yahoo.com (H. Zhong).
[1] These authors contributed equally to this work.

0014-5793/$36.00 © 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2009.11.025

2. Materials and methods

2.1. Cell culture and transfection

293T, MCF7, MCF7/c-Ablkd and MCF7/c-Ablkd/WT c-Abl cells were grown in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin. Cells were treated with 10 μM STI571 (Gleevec; Novartis, Basel, Switzerland) for 4 h.

For downregulation of c-Abl, MCF7 cells were stably transfected with pU6-c-Abl-siRNA using Lipofectamine 2000 (Invitrogen) and selected in the presence of G418 3 days after transfection. The selection of the coding sequences for small interfering RNA (siRNA) was based on previous guidelines [24]. siRNA oligos directed at the same nucleotides were synthesized and were ligated into pU6 vector(Invitrogen) using *BamH*I and *EcoR*I. For c-Abl rescue, pc-Abl rescue cDNA plasmid was made by substitution at positions 330–331 of c-Abl cDNA based on the pcDNA3-Flag-c-Abl plasmid using quick-change mutagenesis kit (Invitrogen).

Vectors and Epitope Tagging of Flag-tagged c-Abl, His-tagged c-Abl, Myc-tagged c-Abl, Flag-tagged MAVS, Myc-tagged MAVS and GST-tagged MAVS were expressed by cloning the genes into the pcDNA3-based vector (Invitrogen).

2.2. Immunoprecipitation and immunoblot analysis

Cell lysates were prepared in lysis buffer (50 mM Tris–HCl [pH 7.5], 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 10 mM sodium fluoride, 10 mg/ml aprotinin, 10 mg/ml leupeptin, and 10 mg/ml pepstatin A) containing 1% Nonidet P-40. Soluble proteins were subjected to immunoprecipitation with anti-Flag (M2, Sigma), anti-Myc (Santa Cruz), or anti-mouse IgG antibody (Sigma). An aliquot of the total lysate (5%, v/v) was included as a control. Immunoblot analysis was performed with anti-Myc, HRP-conjugated anti-Flag (Sigma), anti-c-Abl (Santa Cruz), anti-α -Tubulin (Sigma), anti-P-Tyr (Cell Signaling) and anti-MAVS (Abcam). The antigen–antibody complexes were visualized by chemiluminescence (PerkinElmer Life Sciences).

2.3. Protein binding assays

In direct binding assays, immunoprecipitates were separated by SDS–PAGE and then blotted onto nitrocellulose membranes. Membranes were subsequently incubated with purified GST-fusion proteins for 2 h at room temperature. The GST-fusion proteins were electrophoretically transferred to nitrocellulose then probed with anti-GST antibody.

2.4. Luciferase reporter assays

MCF7 cells were transfected with 0.2 μg of the Luciferase reporter pNF-κB-LUC, IFN-β-LUC or IRF3-LUC plus 0.02 μg of the internal control reporter pCMV-LacZ, with or without various amounts of MAVS expression vector. Transfected cells were collected and Luciferase activity was assessed. All experiments were repeated at least three times.

3. Results

3.1. Interaction between MAVS and c-Abl in vivo and in vitro

We previously noted in a yeast two-hybrid screening that MAVS associates with c-Abl (data not shown). To confirm the yeast two-hybrid analysis, Flag-tagged MAVS or Flag-tagged GP73 was transfected into 293T cells together with His-tagged c-Abl, and a coimmunoprecipitation experiment was performed (Fig. 1A). His-tagged c-Abl was detected in the anti-Flag immunoprecipitation from cells cotransfected with Flag-MAVS, but not with a control Flag-tagged GP73 protein. To confirm the binding of MAVS to c-Abl, 293T cells were cotransfected with plasmids expressing Flag-c-Abl and Myc-MAVS, immunoblot analysis of anti-Flag immunoprecipitates with anti-Myc antibody showed a significant association between Flag-c-Abl and Myc-MAVS (Fig. 1B). This observation substantiates the yeast two-hybrid analysis and establishes an interaction between MAVS and c-Abl. The specificity of the interaction between MAVS and c-Abl was also confirmed by coimmunoprecipitation analysis using normal serum (IgG). Importantly, endogenous MAVS was found to be specifically coimmunoprecipitated with endogenous c-Abl (Fig. 1C).

To rule out an indirect association mediated by other components in the cell lysates, anti-Flag immunoprecipitates prepared from cells expressing Flag-c-Abl were subjected to SDS–PAGE and then blotted onto a nitrocellulose membrane. After incubation with soluble GST–MAVS fusion protein, the nitrocellulose membrane was treated with an HRP-anti-GST antibody. The results showed that c-Abl binds to MAVS directly (Fig. 1D, top). As a control, Flag-c-Abl does not bind to GST (Fig. 1D, middle). Therefore, we conclude that MAVS binds to c-Abl.

3.2. Mapping of the c-Abl binding domains of MAVS

To determine which region(s) of MAVS protein was responsible for mediating the interaction with c-Abl, three mutants containing truncated forms of MAVS lacking either the CARD-like domain (residues 10–77), the proline-rich region (residues 103–152) or the transmembrane domain (residues 514–535) were constructed and overexpressed together with c-Abl. Fig. 2 shows that Myc-tagged c-Abl interacted with full-length and Pro-deleted mutant MAVS but not the TM-deleted mutant or Card-deleted Mutant MAVS. Thus, the interaction with c-Abl is specific for the Card and TM domains of MAVS.

3.3. C-Abl modulates MAVS-mediated induction of IFN-β

To test whether this interaction has functional relevance, the effects of c-Abl on MAVS-mediated type-I IFN activation were explored directly. First, a MCF7 c-Abl knockdown (MCF7/c-Ablkd) clone was generated by stably transfecting a U6 promoter driver plasmid containing c-Abl specific siRNA in the presence of G418. Immunoblotting using anti-c-Abl antibody showed reduced c-Abl expression in MCF7 c-Abl siRNA transfection cells (Fig. 3A). A silent nucleotide substitution in the RNAi region of c-Abl cDNA (pc-Abl-rescue) that was designed to be immune to the interference by sic-Abl was constructed. Stable clone of pc-Ablrescue into the MCF7/c-Ablkd cells expressed Flag-tagged c-Abl with slower mobility in SDS–PAGE (Fig. 3A). Immunoblotting using anti-MAVS antibody showed that these three cell clones do not differ in the total amount of endogenous MAVS.

MCF7, MCF7/c-Ablkd and MCF7/c-Ablkd/WT c-Abl cells were transfected with increasing amounts of expression vectors for MAVS together with an IFN-β Luciferase reporter as well as pCMV-LacZ as an internal control. Thirty-six hours after transfection, the Luciferase activity was measured and normalized based on β-galactosidase activity. As is shown in Fig. 3B, overexpression of MAVS in MCF7 cells potently activated the IFN-β promoter, the extent of activation increased with increasing amounts of expressed MAVS, suggesting that MAVS activated the induction of IFN-β in a dose-dependent manner. MCF7/c-Ablkd cells showed significantly attenuated MAVS-induced IFN-β activation compared with MCF7 or MCF7/c-Ablkd/WT c-Abl cells (Fig. 3B).

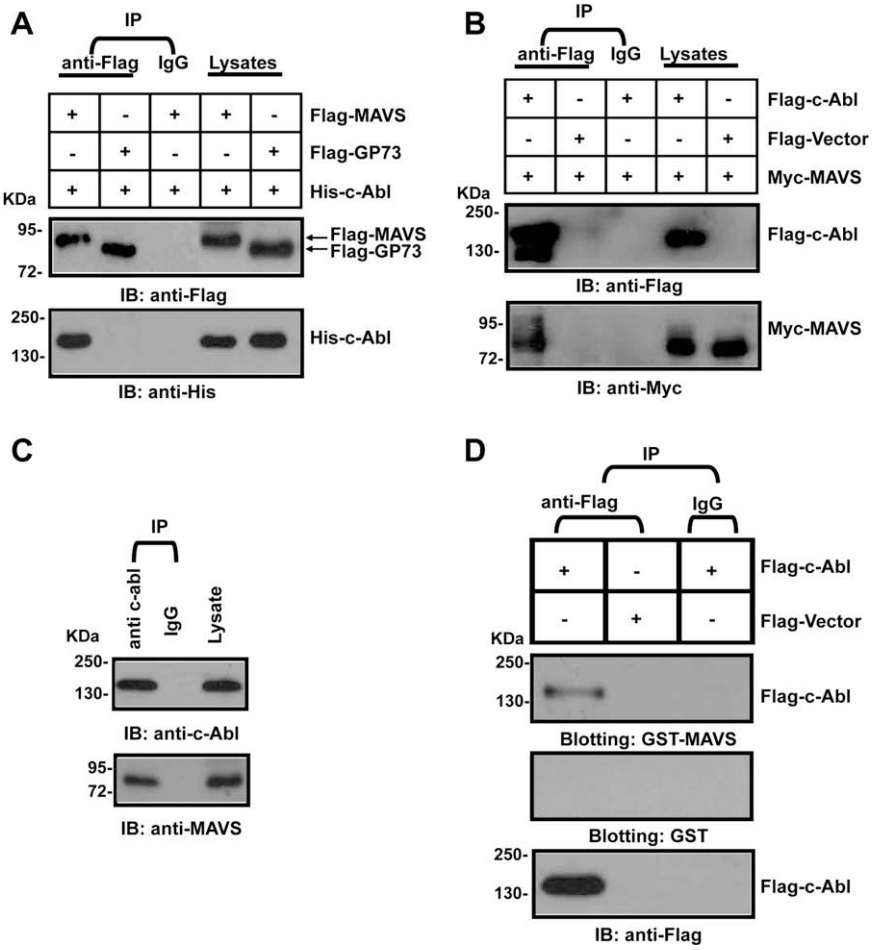

Fig. 1. MAVS associates with c-Abl in vivo and in vitro. (A) 293T cells were cotransfected with His-c-Abl expression plasmids, Flag-MAVS or Flag-GP73, and anti-Flag or IgG immunoprecipitates were analyzed by immunoblotting with anti-His or anti-Flag antibody. (B) 293T cells were cotransfected with Flag-c-Abl and Myc-MAVS expression plasmids or Flag-vector, and anti-Flag or IgG immunoprecipitates were analyzed by immunoblotting with anti-Myc or anti-Flag antibody. (C) Lysates from 293T cells were subjected to immunoprecipitation with anti-c-Abl or IgG, fractionated by SDS–PAGE, and subsequently analyzed by immunoblotting with anti-MAVS antibody. (D) Anti-Flag or IgG immunoprecipitates prepared from cells transfected with Flag-c-Abl or Flag-vector expressing plasmids were subjected to SDS–PAGE and blotted onto nitrocellulose membrane. The nitrocellulose membrane was incubated with soluble GST-MAVS or GST for 2 hr and then analyzed with anti-GST or anti-Flag antibody.

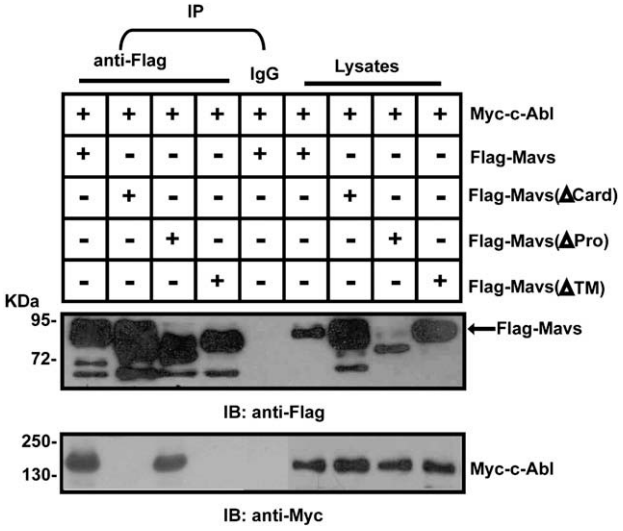

Fig. 2. Mapping of the c-Abl binding domains of MAVS. 293T cells were cotransfected with Myc-c-Abl and Flag-MAVS expression plasmid or Flag-MAVS mutants, anti-Flag immunoprecipitates were analyzed by immunoblotting with anti-Myc or anti-Flag antibody.

Signaling in response to the synthetic viral double-stranded DNA (dsDNA) analogue poly (dAT:dAT) and synthetic viral double-stranded RNA (dsRNA) analogue poly (I:C) are mediated both at the cell membrane through Toll-like receptor 3 (TLR3) and in the cytoplasm by means of direct binding to the RLH molecules to activate MAVS. We delivered poly(dAT:dAT) and poly(I:C) into the cytoplasm by transfection into MCF7, MCF7/c-Ablkd and MCF7/c-Ablkd/WT c-Abl cells, in accordance with previous results, MCF7/c-Ablkd showed significantly attenuated IFN-β activation in response to cytosolic DNA and RNA compared with MCF7 or MCF7/c-Ablkd/WT c-Abl cells (Fig. 3C).

We then assessed if endogenous c-Abl was sufficient to induce the same regulation upon virus infection. IFN-β protein levels using ELISA were monitored in MCF7 cells infected with VSV which activated RIG-I and MAVS for type-1 interferon production. Fig. 3D shows that c-Abl chemical inhibitor STI571 significantly attenuates the ability of VSV to produce IFN-β, ST1571 also severely impaired MAVS-mediated IFN-β production. Taken together, these results showed that c-Abl is required for MAVS-mediated IFN-β activation.

3.4. c-Abl regulates MAVS-mediated induction of NF-κB and IRF3

Both pathways that activate NF-κB and IRF3 lead to IFN-β transcription. We thus attempt to analyze the function of c-Abl

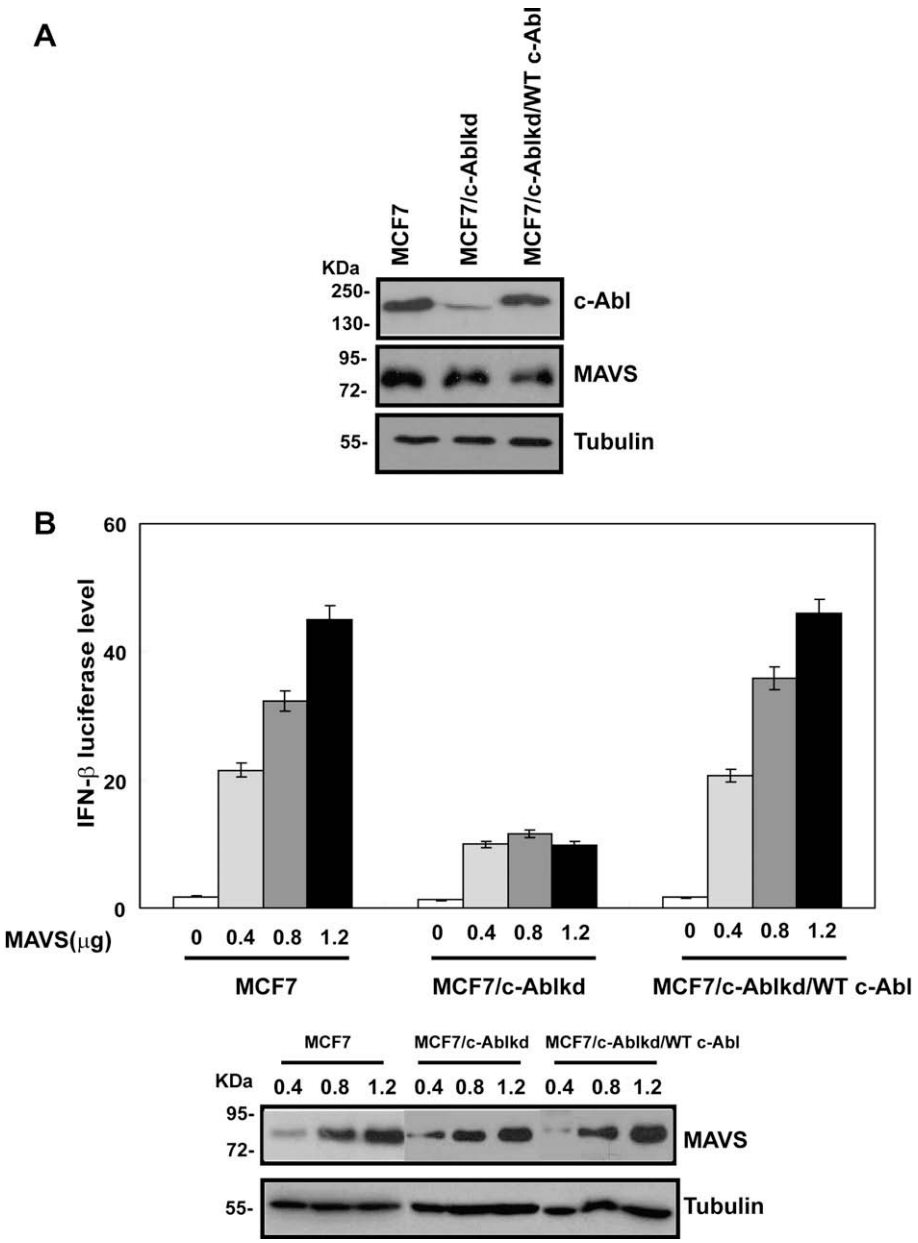

Fig. 3. c-Abl modulates MAVS-mediated induction of IFN-β. (A) Whole cells lysates from MCF7, MCF7/c-Abl kd and MCF7/c-Abl kd/WT c-Abl cells were analyzed by immunoblotting with anti-c-Abl and anti-MAVS antibody, Tubulin was used as equal loading control. (B) MCF7, MCF7/c-Ablkd and MCF7/c-Ablkd/WT c-Abl cells were transfected with increasing amount of MAVS expression vector together with IFN-β-LUC. The LUC activity was measured 36 h later and normalized for transfection efficiency. The error bar represents S.D. from the mean value of duplicated experiments. Whole cells lysates from MCF7, MCF7/c-Abl kd and MCF7/c-Abl kd/WT c-Abl cells were analyzed by immunoblotting with anti-MAVS antibody, Tubulin was used as equal loading control. (C) MCF7, MCF7/c-Ablkd and MCF7/c-Ablkd/WT c-Abl cells were transfected with poly(I:C) or poly(dAT:dAT) together with IFN-β-LUC. The LUC activity was measured 36 h later and normalized for transfection efficiency. The error bar represents S.D. from the mean value of duplicated experiments. (D) IFN-β-LUC was transfected together with Flag-MAVS into MCF7 cells or was transfected into VSV-infected MCF7 cells with or without STI treatment, supernatant IFN-β protein level was measured 36 h later.

on MAVS-mediated NF-κB and IRF3 activation. MCF7 or MCF7/c-Ablkd cells were transfected with increasing amounts of expression vectors for MAVS together with an NF-κB or IRF3 Luciferase reporter as well as pCMV-LacZ as an internal control. Thirty-six hours after transfection, the Luciferase activity was measured and normalized based on β-galactosidase activity. As is shown in Fig. 4A and B, overexpression of MAVS in MCF7 cells potently activates the NF-κB or IRF3 promoter, while overexpression of MAVS in MCF7/c-Ablkd cells showed a significant lower NF-κB or IRF3 activation. Collectively, these results suggest that c-Abl contributes to RIG-I-mediated signaling through its association with MAVS.

3.5. C-Abl phosphorylates MAVS

The association between MAVS and c-Abl suggests that MAVS could be a new substrate for this tyrosine kinase. This notion is supported by several lines of evidence. First, Flag-MAVS was coexpressed with Myc-c-Abl, and the whole cell lysates were immunoprecipitated with anti-Flag antibody. Immunoblotting of the Flag immunoprecipitates with a phosphotyrosine-specific antibody (anti-P-Tyr) indicated that MAVS was phosphorylated by c-Abl (Fig. 5). Second, Flag-MAVS was coexpressed with Myc-tagged kinase inactive c-Abl (K290R) and analyzed for phosphorylation. Immunoblotting analysis demonstrated that Flag-MAVS was phos-

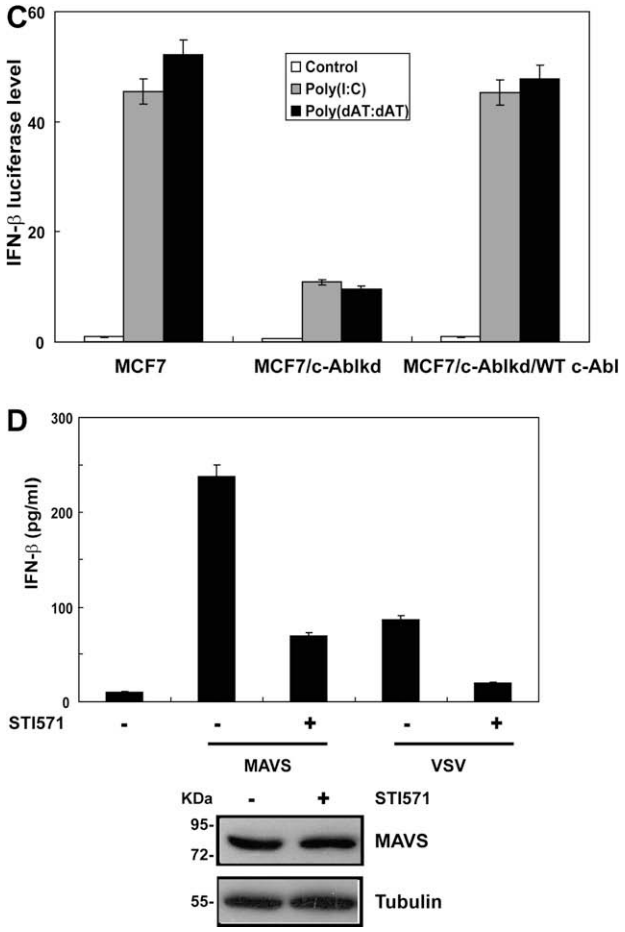

Fig. 3 (*continued*)

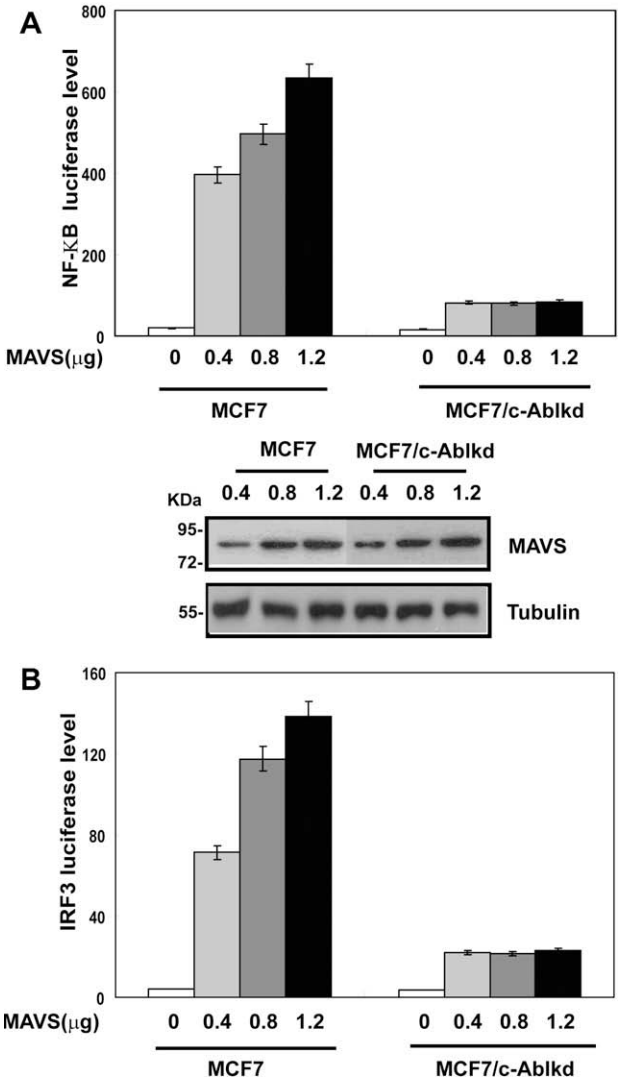

Fig. 4. c-Abl regulates MAVS-mediated induction of NF-κB and IRF3. (A and B) MCF7 and MCF7/c-Ablkd cells were transfected with increasing amount of MAVS expression vector together with NFκB-LUC or IRF3-LUC. The LUC activity was measured 36 h later and normalized for transfection efficiency. The error bar represents S.D. from the mean value of duplicated experiments. Whole cells lysates from MCF7 and MCF7/c-Abl kd cells were analyzed by immunoblotting with anti-MAVS antibody, tubulin was used as equal loading control.

phorylated by WT c-Abl, but not by c-Abl (K290R) (Fig. 5). In concert with these findings, treatment with STI571 inhibited MAVS phosphorylation mediated by overexpressed Flag-c-Abl in 293T cells (Fig. 5).

4. Discussion

Detection of pathogenic nucleic acids by the innate immune system is brought about by several components, including transmembrane TLRs, NLRs, the cytoplasmic RNA helicase RIG-I and the DNA-binding sensor DAI [25]. In this study, we showed that impairment of c-Abl function by either pharmacological inhibition or c-Abl interference RNA strategies effectively suppressed MAVS-stimulated, RIG-I-dependent transcriptional activation of IRF3, NF-κB and IFN-β. This suggests that c-Abl participation in antiviral immune mechanisms is of biological significance. We also found that c-Abl could physically associate with WT MAVS in vivo and in vitro, whereas it failed to interact with different MAVS mutants that lacked the card domain or transmembrane domain. These results demonstrated functional specificity of different MAVS domains in antiviral signaling mediated by MAVS and c-Abl.

Concerning the cellular distribution of c-Abl in the signaling pathway, c-Abl is a ubiquitously expressed non-receptor tyrosine kinase that distributes in both the cytoplasm and the nucleus. Previous studies suggest that nuclear c-Abl inhibits IκB-α degradation by inducing its phosphorylation, which eventually led to NF-κB inactivation. On the contrary, cytoplasmically localized Bcr-Abl can in fact potently activate NF-κB, which has been demonstrated to be essential for Bcr-Abl to induce transformation. Given the fact the MAVS resides in the mitochondria compartment, we suggest that cytosolic c-Abl may play a broader role in innate defense mechanisms.

Numerous studies have indicated that an important step downstream of PRRs is the assembly of multicomponent signaling complexes that activate critical kinases. These signaling complexes serve as structural platforms to assemble downstream signaling molecules. The complexes have been shown to contain TRIF or MAVS (depending pathogenic agent), TANK (TRAF family member-associated NF-KB activator), TBK1 and c-Src [26–28]. In this study, we present novel results showing that c-Abl binds to MAVS through the MAVS card and transmembrane domain, the underlying mechanisms responsible for c-Abl binding to MAVS are not known. Our unpublished data showed extensive MAVS phosphorylation upon virus infection, Fig. 5 shows that MAVS could be phosphorylated by WT c-Abl. In conclusion, our results provide further insight into the role of c-Abl in sensing of intracellular RNA and DNA virus. As such, our results may have implications for antiviral strategies.

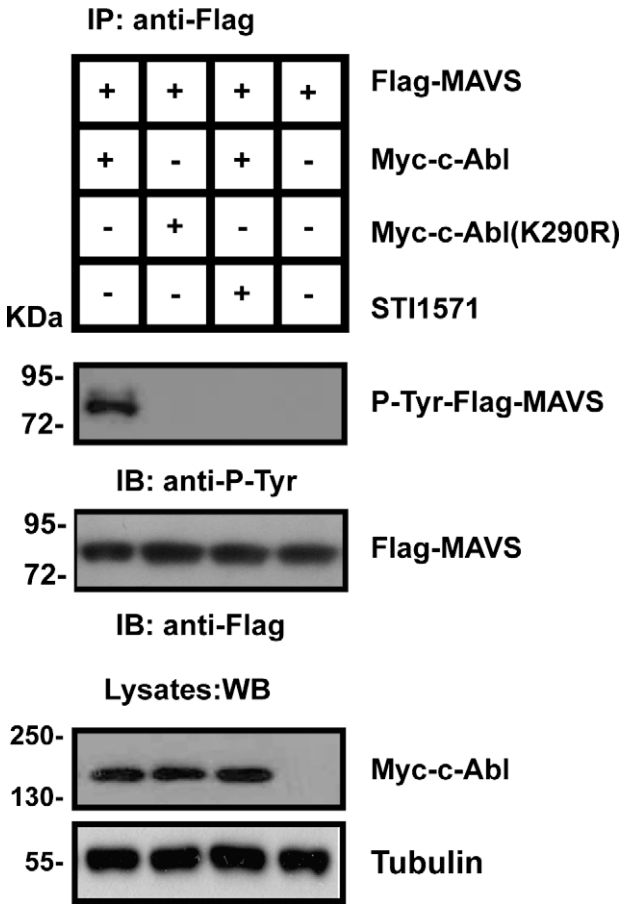

Fig. 5. c-Abl phosphorylates MAVS. 293T cells were transfected with indicated vectors and treated with or without 10 μM STI571 for 12 h. Anti-Flag immunoprecipitates were analyzed by immunoblotting with anti-P-Tyr or anti-Flag antibody.

Acknowledgments

This work was supported by Grants 30772605, 30700413, 30870500 and 30871276 from Natural Science Foundation of China and was supported by Grant 7092081 from Beijing Natural Science Foundation.

Reference

[1] Pendergast, A.M. (2002) The Abl family kinases: mechanisms of regulation and signaling. Adv. Cancer Res. 85, 51–100.
[2] Van Etten, R.A. (1999) Cycling, stressed-out, and nervous: cellular functions of c-Abl. Trends Cell Biol. 9, 179–186.
[3] Plattner, R., Irvin, B.J., Guo, S., Blackburn, K., Kazlauskas, A., Abraham, R.T., York, Y.D. and Pendergast, A.M. (2003) A new link between the c-Abl tyrosine kinase and phosphoinositide signaling through PLC-gamma1. Nat. Cell Biol. 5, 309–319.
[4] Nagar, B., Hantschel, O., Young, M.A., Scheffzek, K., Veach, D., Bornmann, W., Clarkson, B., Superti-Furga, G. and Kuriyan, J. (2003) Structural basis for the autoinhibition of c-Abl tyrosinekinase. Cell 112, 859–871.
[5] Welch, P.J. and Wang, J.Y.J. (1993) A C-terminal protein-binding domain in the retinoblastoma protein regulates nuclear c-Abl tyrosine kinase in the cell cycle. Cell 75, 779–790.
[6] Yukio, N., Raelene, J.G. and Steve, G. (2002) NF-κB1 can inhibit v-Abl-induced lymphoid transformation by functioning as a negative regulator of Cyclin D1 expression. Mol. Cell. Biol. 22, 5563–5574.
[7] Hidehiko, K., Linghu, N. and Zhi-Min, Y. (2002) Inactivation of NF-κB - dependent cell survival, a novel mechanism for the proapoptotic function of c-Abl. Mol. Cell. Biol. 22, 6079–6088.
[8] Kawai, T. and Akira, S. (2006) Innate immune recognition of viral infection. Nat. Immunol. 7, 131–137.
[9] Perry, A.K., Chen, G., Zheng, D., Tang, H. and Cheng, G. (2005) The host type I interferon response to viral and bacterial infections. Cell Res. 15, 407–422.
[10] Akira, S. and Takeda, K. (2004) Toll-like receptor signaling. Nat. Rev. Immunol. 4, 499–511.
[11] Meylan, E. and Tschopp, J. (2006) Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. Mol. Cell 22, 561–569.
[12] Martinon, F. and Tschopp, J. (2005) NLRs join TLRs as innate sensors of pathogens. Trends Immunol. 26, 447–454.
[13] Jörg, H.F., Richard, L.F., Dana, J.P. and Stephen, E.G. (2006) Nod-like proteins in immunity, inflammation and disease. Nat. Immunol. 7, 1250–1257.
[14] Inohara, N. and Nunez, G. (2003) NODs: intracellular proteins involved in inflammation and apoptosis. Nat. Rev. Immunol. 3, 371–382.
[15] Inohara, N., Chamaillard, M., McDonald, C. and Nunez, G. (2005) NOD-LRR proteins: role in host–microbial interactions and inflammatory disease. Annu. Rev. Biochem. 74, 355–383.
[16] Kumar, H., Kawai, T., Kato, H., Sato, S., Takahashi, K., Coban, C., Yamamoto, M., Uematsu, S., Ishii, K.J. and Takeuchi, O. (2006) Essential role of IPS-1 in innate immune responses against RNA viruses. J. Exp. Med. 203, 1795–1803.
[17] Kato, H., Sato, S., Yoneyama, M., Yamamoto, M., Uematsu, S., Matsui, K., Tsujimura, T., Takeda, K., Fujita, T. and Takeuchi, O. (2005) Cell type specific involvement of RIG-I in antiviral response. Immunity 23, 19–28.
[18] Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., Yamamoto, M., Matsui, K., Uematsu, S., Jung, A., Kawai, T. and Ishii, K.J. (2006) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441, 101–105.
[19] Kawai, T., Takahashi, K. and Sato, S. (2005) An adaptor triggering RIG-1 and Mda5-mediated type I interferon induction. Nat. Immunol. 6, 981–988.
[20] Yoneyama, M., Kikuchi, M. and Natsukawa, T. (2004) The RNA helicase RIG-1 has an essential function in double-stranded RNA-induced innate antiviral responses. Nat. Immunol. 5, 730–737.
[21] Rashu, B.S., Lijun, S., Chee-Kwee, E. and Zhijian, J.C. (2005) Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-κB and IRF3. Cell 122, 669–682.
[22] John, H., Rongtuan, L., Peyman, N. and Suzanne, P. (2006) MasterCARD-a priceless link to innate immunity. Trends Mol. Med. 12, 2–10.
[23] Liang-Guo, X., Yan-Yi, W., Ke-Jun, H., Lian-Yun, L., Zhonghe, Z. and Hong-Bing, S. (2005) VISA is an adaptor protein required for virus-triggered IFN-β signaling. Mol. Cell 19, 727–740.
[24] Xiang, H., Congwen, W., Ting, S., Jing, Y., Yanhong, Z., Qingjun, M., Wei, S. and Hui, Z. (2009) Proliferating cell nuclear antigen destabilizes c-Abl tyrosine kinase and regulates cell apoptosis in response to DNA damage. Apoptosis 14, 268–275.
[25] Akinori, T., ZhiChao, W., Myoung, K.C., Hideyuki, Y., Hideo, N., Tatsuma, B., Yan, L., Makoto, M., Tatsuhiko, K., Kenya, H., Yusuke, O. and Tadatsugu, T. (2007) DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature 448, 501–505.
[26] Ingvild, B.J., Thuy, T.N., Bjarte, B., Katherine, A.F. and Marit, W.A. (2009) The tyrosine kinase c-Src enhances RIG-I (retinoic acid-inducible gene I)-elicited antiviral signaling. J. Biol. Chem. 284, 19122–19131.
[27] Beichu, G. and Genhong, C. (2007) Modulation of the interferon antiviral response by the TBK1/IKKi adaptor protein TANK. J. Biol. Chem. 282, 11817–11826.
[28] Ryzhakov, G. and Randow, F. (2007) SINTBAD, a novel component of innate antiviral immunity, shares a TBK1-binding domain with NAP1 and TANK. EMBO J. 26, 3180–3190.